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P-1

Incidence of clonal hematopoiesis-related mutations in low-count monoclonal B-cell lymphocytosis (MBLlo) subjects

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Background

Deep genomic sequencing of normal subjects revealed that during human aging, the expansion of 1 or more hematopoietic stem cells (HSCs) will result in clones that will contribute more than others to the production of mature blood cells. Age-related clonal hematopoiesis (CH) is defined as the expansion of HSC clones harboring specific, disruptive and recurrent genetic variants in individuals without clear diagnosis of hematologic malignancies. Sooner or later, these age-related CH-related mutations can increase the risk for leukemia. Then, age-related CH may also be associated with the expansion of B-cell clones with a CLL phenotype, as it happens in low-count monoclonal B-cell lymphocytosis (MBLlo), which has increasing prevalence in adulthood. For that reason, we analyze the existence of mutations in CH-related genes in leukocytes from MBLlo adults and age- and sex-matched healthy donors.

Material and methods

Monocytes, neutrophils and CD4+ T-cells were sorted from 105 donors with CLL-like MBLlow [69 males and 36 females, median age (range): 58 (30-89) and 56 (33-95); respectively] and 56 healthy donors [39 males and 17 females, median age (range): 60 (30-88) and 64 (21-90); respectively]. DNA from sorted cell populations was purified and subjected to a custom capture-based panel for targeted sequencing of 239 genes related to CH, through Illumina NovaSeqX platform. Sequenced data were aligned to the reference genome and filters were applied to remove SNPs, sequencing mistakes and germline variants from the analysis. Only those mutations with VAF<0.45 (passenger mutations) or VAF>0.55 (driver mutations) and found in all cell populations were considered.

Results

Mutations in genes involved in CH were detected in similar proportion of subjects from both cohorts (MBLlo: 15% -16/105- vs healthy: 12%-7/56-; P=0.814), however MBLlo subjects carrying different CH-related mutations were significantly younger than healthy donors carrying other CH-related mutations (median age: 61y vs 75y, P=0.025; respectively).

The mutations found were different and heterogeneous in both cohorts. Twenty-three genes -RTEL1, CBL, CSF1R, CBLC, ABCB6, PMS2, CSMD1, ASXL1, GNAS, PML, FGFR1, KMT2D, IKZF3, TET2, KRAS, ATM, SAMD9, DNMT3A, NBEAL2, JAK2, PPM1D, FANCA and FAT1- showed somatic mutations -missense (n=14), deletions (n=9) and insertions (n=2)-. Only GNAS (n=5), CBLC (n=2), PMS2 (n=2), and ATM (n=2) had >1 mutation. GNAS p.P459R was detected in five MBLlo subjects; CBLC p.P435S in one MBLlo subject and one healthy donor. PMS2 and ATM had different alterations in both cohorts: PMS2 p.E431_K432insHVDSQE in an MBLlo subject and p.L729Qfs*6 in a healthy donor; ATM p.S707P in an MBLlo subject and p.L1420F in a healthy donor. To note, those MBLlo subjects carrying the GNAS mutation did not show a myelodysplasia-associated phenotype in blood.

Conclusions

We found a similar frequency of cases with CH-related mutations in subjects with MBLlo and age- and sex-matched healthy donors (around 15%). Interestingly, such CH related mutations appeared in MBLlo cases who were significantly younger than the healthy. Likewise, the genes found to be altered in both cohorts were different. Further research with an extended series is needed to gain greater insight into the pathogenesis of CH in MBLlo subjects.

P-2

Characteristic patterns of the levels of 5-methylcytosine oxidation products and vitamin C in chronic lymphocytic leukemia (CLL) and monoclonal B-cell lymphocytosis (MBL)

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Background
Monoclonal B-cell lymphocytosis (MBL) is a hematological premalignant condition characterized by the presence of a clonal B-cell population with a count smaller than 5 x 10^9 /L. Based on the clonal B-cell count, MBL can be categorized into two subtypes: low-count MBL and high-count MBL. Several studies have demonstrated that approximately 1-2 % of high-count MBL yearly can progress to chronic lymphocytic leukemia (CLL), while low-count MBL rarely progress to CLL. The leukemia cases account for 2.5 % of all cancer events, and about 30-40 % of them are CLL. Accordingly, CLL is known as the most frequent type of leukemia in adult patients, and not only genetic but also epigenetic alterations can be involved in its development. Many studies have proven that in various cancers, including CLL, the levels of 5-hydroxymethylcytosine are decreased. Moreover, reduced vitamin C levels have also been found, which is significant because vitamin C plays a role as a cofactor for TET enzymes, catalyzing the reaction of oxidation of 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt) and further to 5-formylcytosine (5-fCyt) and 5-carboxylcytosine (5-caCyt).

Material and methods
In our studies, we analyzed the samples from 56 MBL patients, 134 CLL patients, and 92 donors with excluded hematological malignancies. We performed analysis of 5-mCyt, 5-hmCyt, 5-fCyt, 5-caCyt, and 5-hydoxymethyluracil (5-hmUra) levels on the whole-genome level using isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry with stable isotope-labeled internal standards (2D-UPLC-MS/MS). Furthermore, we analyzed vitamin C concentrations in blood plasma by UPLC-UV and intracellular vitamin C content in isolated peripheral blood mononuclear cells by UPLC-MS.

Results
There were statistically significant differences in the levels of 5-mCyt and 5-fCyt in all studied groups. We have also found different 5-hmCyt and 5-caCyt levels between the control group and MBL and the control group and CLL. Interestingly, the levels of 5-hmUra were considerably increased in CLL compared to MBL and controls. The highest vitamin C content in cells was observed in CLL patients and the level was significantly higher than in controls.

Conclusions
In conclusion, our results indicate characteristic patterns in the levels of 5-mCyt and its oxidation products in the studied groups. Changes in the epigenetic landscape may help predict MBL progression to CLL in the future.

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P-3

The frequency of TP53 alterations and the significance of low-burden variants in a single center Hungarian CLL cohort

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Background
Tumor protein 53 (TP53) is the 'guardian of the genome.' TP53 gene alterations were studied since the beginning of cancer genetics. In accordance with the classic tumor suppressor mechanism, alterations affecting both alleles resulting in complete biallelic loss of function or dominant negative single allele mutations are common, but single allele gain-of-function mutations were also described in the pathomechanism.

Material and methods
In our laboratory, between 2018 and 2023, the deletion of the TP53 gene (TP53del) was detected by fluorescence in situ hybridization (FISH, with Vysis CEP17/TP53 probe), and the mutation of the TP53 gene (TP53mut) by next-generation sequencing (NGS, Agilent or Paragon Cleanplex) in 523 chronic lymphoid leukemia (CLL) and additionally 244 multiple myeloma (MM) cases.

Result
For TP53 NGS, we determined the reporting threshold (limit of detection - LOD) based on the comparison of two NGS kits at 4% based on multiple independent NGS runs. In CLL (n=523), 85.85% showed no TP53 alterations, while 1.91% (n=10) showed only TP53 deletion (TP53del), 6.12% (n=32) showed only TP53 mutation (TP53mut), and 6.12% (n=32) showed double hits. We detected 29 TP53 variants between 4-10% in frequency among 22 patients, of which 16 patients had no alterations exceeding 10%. Interestingly, in TP53 positive CLL cases (n=74), 43% (n=32/74) harbored only mutations, while only mutation cases occurred in 14% (n=12/85) of TP53 –altered MM cases ($p<0.0001$). In contrast, the dominance of cases with only TP53 deletion was characteristic for MM [the proportion of MM cases with only TP53del among TP53 affected cases is 48% (41/85) vs. CLL 14% (10/74); ($p<0.0001$)].

Conclusions
In the CLL cohort, as well as in MM, double-hit TP53 involvement is significant. However, gene sequencing alterations are more frequent than chromosomal-level alterations. Therefore, our results suggest that certain TP53 alterations may have different pathogenetic effects in various lymphoid malignancies. Additionally, as stated in the latest guidelines, it is crucial to accurately determine the detection threshold of laboratories. This is essential to ensure that patients carrying only low-burden variants can also have access to targeted therapies.

P-4

Preliminary efficacy and safety of the Bruton tyrosine kinase (BTK) degrader BGB-16673 in patients with relapsed or refractory (R/R) CLL/SLL: Results from the phase 1 BGB-16673-101 study

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Background
BTK inhibitors are approved for CLL, but intolerability and treatment resistance can limit their use. BGB-16673 is a heterobifunctional small molecule that induces BTK degradation via ubiquitination. In preclinical models, BGB-16673 degraded wild-type and mutant BTK proteins resistant to covalent (cBTKis) and noncovalent BTK inhibitors (ncBTKis), leading to tumor regression. BGB-16673 is currently being evaluated in phase 1 studies. Updated results from patients with CLL/SLL in the phase 1 portion of the open-label first-in-human, BGB-16673-101 study (NCT05006716) are presented.

Material and Methods
Eligible patients must have R/R CLL/SLL (≥2 prior therapies), an ECOG performance status of 0-2, and adequate end-organ function. In the US, EU, and Australia, patients must have previously received a cBTKi. BGB-16673 was dosed once daily orally in 28-day cycles. Dose escalation using a Bayesian optimal interval design with 6 dose levels (50-600 mg once daily) was planned. Primary objectives were to assess safety/tolerability per CTCAE v5.0 and iwCLL hematologic toxicity criteria and establish the maximum tolerated dose (MTD) and recommended phase 2 dose. Dose-limiting toxicities (DLTs) were assessed in the first 4 weeks (cycle 1). Response was assessed per iwCLL 2018 criteria (or Cheson et al, 2014 for SLL), with first assessment after 12 weeks of treatment.

Results
As of November 9, 2023, 42 patients with CLL were enrolled (median age, 70 years; range, 50-91) and 39 were treated (50 mg, n=1; 100 mg, n=5; 200 mg, n=15; 350 mg, n=14; 500 mg, n=4). Treated patients had a median of 4 prior therapies (range, 2-8), including cBTKis (n=37; 95%), BCL2 inhibitors (n=34; 87%), and ncBTKis (n=10; 26%). Of tested patients, 54% (20/37) had del(17p) and/or TP53 mutation, 87% (27/31) had unmutated IGHV, and 43% (12/28) had ≥3 karyotypic abnormalities. Median follow-up time was 3.3 months (range, 0.1-16.7). One DLT occurred in 1 patient (200 mg; grade 3 maculopapular rash on day 27; after 5-day dose hold, assigned dose was reinitiated with persistent grade 1 rash). MTD was not reached. The most common TEAEs were contusion (31%; no grade ≥3), fatigue (31%; no grade ≥3), diarrhea (26%; no grade ≥3), and neutropenia (23%; grade ≥3, 18%). One patient (500 mg) had a TEAE of grade 3 hypertension. No atrial fibrillation was observed. TEAEs led to death in 2 patients (septic shock and pneumonia; neither was considered related to treatment), treatment discontinuation in 2 additional patients (subdural hemorrhage and thyroid cancer), and dose reduction in 1 patient (grade 2 arthralgia). Thirty-five of 39 patients (90%) remain on therapy (4 discontinuations: progressive disease, n=1; AEs, n=3). For 24 response-evaluable patients, the ORR was 67%, with all but 1 response ongoing. Responses were seen at the lowest dose, in patients previously treated with cBTKi (n=16) and ncBTKi (n=2), and in patients with and without BTK mutation.

Conclusions
Emerging data from this ongoing, first-in-human study of the novel BTK degrader BGB-16673 demonstrate a tolerable safety profile and antitumor activity in heavily pretreated patients with CLL/SLL, including those with BTK inhibitor-resistant mutations.

P-5

Results from the phase 1 study of the novel BCL2 inhibitor sonrotoclax in combination with zanubrutinib for relapsed / refractory (R/R) CLL / SLL show deep and durable response

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Background
Sonrotoclax (BGB-11417), a next-generation BCL2 inhibitor, is a more selective and potent inhibitor of BCL2 than venetoclax in biochemical assays. Zanubrutinib, a next-generation BTK inhibitor, has shown improved PFS and tolerability, including fewer cardiac AEs than ibrutinib in a randomized study of patients with R/R CLL/SLL and is approved for CLL. Updated safety and efficacy data for patients with R/R CLL/SLL treated with sonrotoclax + zanubrutinib in the ongoing BGB-11417-101 (NCT04277637) study are presented.

Material and Methods
Patients with R/R CLL/SLL received zanubrutinib (320 mg QD or 160 mg BID) 8-12 weeks before starting sonrotoclax (40, 80, 160, 320, or 640 mg QD) with ramp-up to the target dose to mitigate potential tumor lysis syndrome (TLS) risk. Prior BTK inhibitors were allowed if disease progression occurred on treatment. Patients were treated until disease progression or unacceptable toxicity. The primary endpoint was safety CTCAE v5.0. ORR iwCLL 2008 criteria and minimal residual disease assessed in blood by ERIC flow every 24 weeks (uMRD4) were secondary and exploratory endpoints, respectively.

Results
As of Oct 31, 2023, 45 patients with R/R CLL/SLL were enrolled (40 mg, n=4; 80 mg, n=9; 160 mg, n=6; 320 mg, n=20; 640 mg, n=6). Four patients were still in the zanubrutinib lead-in phase and 41 had started sonrotoclax. The median age was 65 years (range, 36-76); 28% of tested patients (11/40) had del(17p), and 72% (13/18) had unmutated IGHV. The median number of prior treatments was 1 (range, 1-3); 7 patients had a prior BTK inhibitor as their last therapy. Median follow-up was 17 months (range, 0.5-32.6). No DLTs occurred, and the MTD had not been reached up to 640 mg. Dose expansion was completed with a recommended phase 2 dose of 320 mg. Any-grade treatment-emergent AEs (TEAEs) in ≥20% of patients were COVID-19 (n=12; 27%), contusion (n=12; 27%), neutropenia (n=12; 27%), diarrhea (n=11; 24%), nausea (n=11; 24%) and fatigue (n=11; 24%). Neutropenia was the most common grade ≥3 TEAE (n=9; 20%). No TLS or atrial fibrillation occurred; no TEAEs led to death, discontinuation, or dose reduction. Sonrotoclax dose holds occurred in 14 patients for a median duration of 7 days, most commonly due to COVID-19 (n=7). For 32 response-evaluable patients, the ORR was 97% (31/32); 1 patient (40 mg) had SD. The complete response (CR) rate was 50% (40 mg, n=1 [25%]; 80 mg, n=4 [50%]; 160 mg, n=4 [67%]; 320 mg, n=5 [56%]; 640 mg, n=2 [40%]). Median time to CR was 9.8 months (range, 5.5-18.2). Of 4 response-evaluable patients with prior BTK inhibitor treatment, 3 achieved PR (n=2) or CR (n=1). All patients treated with sonrotoclax + zanubrutinib (160 mg, 320 mg, or 640 mg) who reached week 48 achieved uMRD4. Treatment is ongoing for all but 1 patient (40 mg) who discontinued due to disease progression.

Conclusions
Efficacy of sonrotoclax + zanubrutinib combination treatment is encouraging, with a 97% ORR and deep responses, including uMRD, in patients with R/R CLL/SLL. This combination has demonstrated a tolerable safety profile across all dose levels tested.

P-6

Combination treatment with sonrotoclax (BGB-11417), a second-generation BCL2 inhibitor, and zanubrutinib, a Bruton tyrosine kinase (BTK) inhibitor, is well tolerated and achieves deep responses in patients with treatment-naive chronic lymphocytic leukemia/small lymphocytic lymphoma (TN-CLL/SLL): data from an ongoing phase 1/2 study

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Background
Sonrotoclax (BGB-11417), a next-generation BCL2 inhibitor, is a more selective and potent inhibitor of BCL2 than venetoclax in biochemical assays. Zanubrutinib, a next-generation BTK inhibitor, improved PFS with fewer cardiac AEs than ibrutinib in patients with CLL/SLL. BGB-11417-101 (NCT04277637) is an ongoing, first-in-human, phase 1/1b dose-escalation/expansion study of patients with B-cell malignancies. Presented here are data from patients with TN CLL/SLL treated with sonrotoclax + zanubrutinib.

Material and Methods
Patients received zanubrutinib (320 mg QD or 160 mg BID) 8 to 12 weeks before starting sonrotoclax with a ramp-up schedule to target doses of 160 or 320 mg QD to mitigate risk of tumor lysis syndrome (TLS). Patients were treated until progression or unacceptable toxicity. TLS was assessed per Howard 2011 criteria. The primary endpoint was safety per CTCAE v5.0; a secondary endpoint was ORR per iwCLL 2008 criteria, and minimal residual disease (uMRD4; <1 CLL cell per 10,000 leukocytes, or <0.01%) in blood by ERIC flow every 24 weeks was an exploratory endpoint.

Results
As of May 21, 2023, 94 patients with TN CLL/SLL were enrolled; 15 patients were still in zanubrutinib lead-in and 79 had started sonrotoclax (160 mg, n=32; 320 mg, n=47). Median follow-up was 8.5 months (range, 0.6-18.2) for all patients, 12.1 months (range, 0.6-18.2) for 160 mg, and 7.0 months (range, 1.1-14.6) for 320 mg. No deaths occurred, and all patients remain on study. Treatment-emergent AEs (TEAEs) in ≥20% of patients who received sonrotoclax + zanubrutinib were contusion (35%), neutropenia (35%), COVID-19 (23%), and diarrhea (23%; grade ≥3 in 1 patient). Neutropenia was the most common grade ≥3 TEAE (17%). No clinical or laboratory TLS occurred. No patients experienced atrial fibrillation. One TEAE (cryptococcal meningitis at 11 weeks) led to treatment discontinuation. Sonrotoclax dose holds occurred in 17 patients (22%; median duration, 11 days [range, 3-37]); 3 patients (4%) had dose reduction. In 56 response-evaluable patients, ORR was 100% (CR: 160 mg, 36% [n=9]; 320 mg, 19% [n=6]). CR rate increased with time; the median time to CR was 10.1 months (range, 5.4-17.1). No progression events were reported in either cohort. Week 24 blood uMRD4 rates were 50% (12/24) for 160 mg and 65% (13/20) for 320 mg. Week 48 blood uMRD4 rates were 73% for 160 mg (11/15) and 100% (1/1) for 320 mg.

Conclusions
Sonrotoclax (160 and 320 mg) + zanubrutinib was well tolerated in patients with TN CLL/SLL. Only 1 patient discontinued treatment and 3 had dose reductions. No TLS was seen. Efficacy is encouraging, with 100% ORR in assessed patients, no PFS events, and high rates of blood uMRD4 occurring early. A phase 3 study assessing this combination is planned.

P-7

Prognostic Impact of High-Risk Genetic Features in CLL Patients Treated with Ibrutinib: A Comparison between Frontline and Subsequent Lines

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Background
Complex karyotype is recognized as a high-risk marker in chronic lymphocytic leukemia (CLL) together with del(17p), del(11q), unmutated immunoglobulin heavy chain variable region (IGHV) status. Ibrutinib, a BTK inhibitor, has demonstrated exceptional efficacy in CLL treatment, irrespective of therapeutic line or genetic profile and it is now considered a first choice, alongside other BTK inhibitors, for high-risk CLL.

Aim
To evaluate the prognostic impact of high-risk genetic features in patients with CLL treated either in the frontline or subsequent lines with ibrutinib.

Methods
We conducted a retrospective study involving 150 patients who underwent treatment with Ibrutinib either as initial therapy (N=81) or in the relapsed/refractory (R/R) setting (N=69) between 2015 and 2023 at the University Hospital of Padua. The patients' cytogenetic and FISH profiles, IGHV mutational status, as well as TP53 and NOTCH1 mutations, were assessed. Overall survival (OS) and progression-free survival (PFS) was calculated. Complex karyotype (CK) and High CK were defined by the presence of 3 and 5 chromosomal abnormalities, respectively.

Results
The median age at enrollment was 72 years (range 43-93), with no differences observed between the first-line and R/R cohorts. Patients in the R/R group received a median of 3 (range 2-6) prior lines of therapy. Regarding genetic characteristics, 25% exhibited a CK, 9% had a High CK, 19% showed del(17p), 19% displayed del(11q), and 25% harbored TP53 mutations, with no statistical differences between patients treated in the first line setting versus subsequent lines. The incidence of unmutated IGHV status was found to be higher in the R/R setting in our population (54% vs. 74%, p=0.03).
Considering the whole population, the median PFS was 49 months while median OS was NR. In multivariate analysis CK (p=0.0056) and treatment line (p=0.0016) were significant predictors of PFS. Instead, only High CK demonstrated a significant association with OS in multivariate analyses (p=0.011). Unmutated IGHV, TP53 mutation, del(17p) and del(11q) did not impact in PFS and OS.
Assessing the impact of high-risk lesions in patients treated in the frontline and subsequent lines, a significant negative impact on PFS emerges for CK (p=0.035) and del(17p) (p=0.016) in the frontline setting, while no impact was observed in R/R patients.

Conclusions
Our findings highlight the negative impact of CK on PFS in patients receiving ibrutinib as first line, while only patients with high CK had a shorter OS likely due to a poor response to subsequent venetoclax-based regimens (Serafin, BJH 2024).

P-8

Development of new preclinical models to investigate response and resistance to therapy in Chronic Lymphocytic Leukemia (CLL) using 3D Bioprinting

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Background

Chronic Lymphocytic Leukemia (CLL) strongly relies on the interactions occurring between CLL cells and the microenvironment, fostering proliferation and drug refractoriness. A deeper understanding about the intrinsic/extrinsic mechanisms driving CLL pathogenesis is needed to enhance new therapies. Nevertheless, current preclinical models have limitations that can be potentially overcome by 3D cell culture systems. We previously demonstrated that 3D bioprinted CLL cells can survive up to 28 days in these in vitro models. The aim of my project is to exploit 3D bioprinting to generate more complex and reproducible preclinical models to perform drug testing in CLL.

Methods

Primary CLL cells and MEC1 CLL cell line were bioprinted in polymer-based hydrogels: CELLINK Laminink 411 (commercial) and 3A1M ink (home-made: methylcellulose, alginate and gelatine). 2D cultured and 3D bioprinted CLL cells were treated with the chemotherapeutic agent Fludarabine and the BCL2 inhibitor Venetoclax for 24 and 72 hours after 1 or 7 days of 3D culture adaptation. To mimic the microenvironment, we co-printed human lymphatic fibroblast (HLF) and bone marrow stromal cells (HS27-A) in GELXA Laminink 411 hydrogel (commercial) and MEC1 cell line in 3A1M ink. We assessed cell viability by Alamar Blue and flow cytometry; and gene expression profile by RT-qPCR and RNA-Seq analysis.

Results

We observed a higher resistance to both chemotherapy and target agents in 3D bioprinted MEC1 cell line after 7 days of 3D culture adaptation as compared to 2D conditions ($p<0.05$). We confirmed this trend by treating 3D bioprinted primary CLL cells (n=7). By RNA-Seq analysis and RT-qPCR validation on 2D and 3D bioprinted cells, we identified the regulation of genes linked to survival and adaptation to the environment (i.e. BAX, CXCR5, IL2RA). Moreover, 3D bioprinted MEC1 cell line showed an upregulation of CD62L and AICDA, genes involved in cell adhesion and adaptive immune response, respectively, possibly playing a significant role in CLL refractoriness and progression. We are currently investigating the involvement of other genes in the mechanism of resistance observed in the 3D biorpinted CLL cells. Moreover, thanks to a dynamic mechanical analysis, we assessed the mechanical properties of our constructs and observed a comparable range of stiffness between the cell-laden scaffold and lymphoid organs (0,5-1,5 Kpascal), suggesting that the mechanical properties of the scaffolds could have a role in the behaviour and response to therapy of CLL cells. In parallel, we are co-printing CLL cells with the lymphoid microenvironment. Preliminary data support the feasibility of this approach, and we are currently performing drug testing under these conditions.

Conclusions

We demonstrated that 3D bioprinting promotes CLL cells viability by affecting genes involved in homing and survival. This adaptation to new culture settings reflects a different response to either standard chemotherapy and target therapies in both CLL cell line and primary cells compared to 2D condition. These results pave the way for the generation of more complex in vitro models that include the microenvironment and controlled mechanical settings for a more robust and more reproducible investigation of the mechanisms that drive CLL onset, progression and relapse.

P-9

Excellent clinical outcome in deeply mutated chronic lymphocytic leukemia upon FCR

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Background
In patients with chronic lymphocytic leukemia (CLL) chemoimmunotherapy (CIT) with fludarabine and cyclophosphamide plus rituximab (FCR) is the standard of care for fit younger patients <65 years of age, who are immunoglobulin heavy chain variable gene (IGHV) mutated without TP53 aberrations. IGHV is considered mutated if it deviates more than 2% from the germline sequence (GS). For most patients, FCR leads to long progression-free survival (PFS; Thompson et al, Blood 2024) although the risk of secondary malignancy and therapy-related myeloid neoplasms is significant (da Cunha-Bang, BJH 2021), and in recent years targeted treatments have replaced CIT for most patients. Thus, it is debated whether there is still a clinical indication for FCR.

Material and methods
In this retrospective study we included patients diagnosed between Jan 2006 and Oct 2021 from a nationwide CLL register, who received FCR as first line treatment. Patients were grouped according to the degree of IGHV GS identity: IGHV unmutated (IGHV-U) GS ≥98%, IGHV intermediately mutated (IGHV-I) GS 94-98%, and IGHV deeply mutated (IGHV-D) GS <94%. Lacking information on PFS, we analyzed time to next treatment (TTNT) or death (event) using the Kaplan-Meier method following patients until next treatment, death, or end of follow-up.

Results
In total, 420 patients received frontline FCR with information on IGHV status in 378 patients, while information on IGHV GS homology was available in 134 patients: 97/134 (72%) patients were IGHV-U, 26/134 (19%) were IGHV-I and 11/134 (8%) were IGHV-D. At time of diagnosis, the 420 patients had a median age of 60 years (IQR 53;65), 69.5% were male, 49% were Binet A, 59% were IGHV-U, and 6% had a 17p deletion. The median time from diagnosis to FCR was only 10 months (IQR 2;27) and the latest patient received FCR in Nov 2021. The median follow-up time from FCR was 7.5 years (IQR 5.7;9.7). As expected, patients with a mutated IGHV demonstrated longer PFS as compared to those with IGHV-U (5-year TTNT 84.6% vs of 51.3%, respectively; p<0.0001). PFS for patients with IGHV-D demonstrated excellent 5-year and 10-year PFS of 100.0% and 83.3% (none receiving 2nd line therapy) as compared with 78.9% and 61.5% in IGHV-I, respectively (p=0.11).

Conclusion
In this retrospective real-world study of PFS in CLL patients treated with frontline FCR, we demonstrate an excellent PFS among a smaller subset of patients with IGHV-D. The study is limited by a small number of patients, which increases the risk of chance findings and likely resulted in the PFS difference between IGHV-D and IGHV-I patients not meeting statistical significance. Even so, the degree of IGHV mutation - rather than IGHV status per se – may identify a group of IGHV-D patients, who still benefit from FCR.

P-10

Prevalence and clinical impact of clonal hematopoiesis of indeterminate potential (CHIP) in chronic lymphocytic leukemia

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Background
Clonal Hematopoiesis of Indeterminate Potential (CHIP) is associated with a variety of different diseases but its prognostic role in of chronic lymphocytic leukemia (CLL) is unclear.

Methods
Tumor genomic DNA was extracted from granulocytes from CLL patients (N=367). Samples were analyzed by targeted next-generation sequencing (NGS), employing a custom panel of recurrently mutated genes in CHIP (N=28) and sequenced by MiSeq and NextSeq550 platforms (Illumina). Statistical analysis was performed using R studio and SPSS software.

Results
A total of 167 (45.5%) patients showed at least 1 CHIP mutation, which significantly correlated with older age (p=0.004). The most frequently mutated genes were DNMT3A in 89 (24.3%) patients, followed by TET2 in 52 (14.2%) and ASXL1 in 10 (2.7%) (Fig1A). At the current median follow-up (13.9 years), CHIP+ patients presented shorter overall survival (OS) compared to CHIP- patients (p=0.04) and this is mainly driven by TET2 mutations, which emerged as an independent predictor of shorter OS (p=0.0076) when adjusted for age, IGHV and TP53 status (Fig1B, 1C). No difference in time to first treatment (TTFT) was observed between CHIP+ and CHIP- patients. The potential clinical impact of CHIP in Richter transformation (RT) was also assessed. CHIP overall did not associate with an increased risk of RT, whereas ASXL1 mutations independently associate with an in increased risk of RT (HR 6.96, 95% CI 1.56-31.05, p=0.01), even when adjusted for TP53 disruption and NOTCH1 mutations (Fig1D, 1E). Subsequently, the clonal evolution of CHIP following therapy was assessed. Longitudinal analysis in 25 patients treated with chemoimmunotherapy (CIT) showed that CIT led to an increase in the number of CHIP mutations and in the variant alle frequency (VAF) of pre-existing mutations (p=0.004). By analyzing CHIP dynamics during BCL2 inhibitors (BCL2i) therapy (N=15), no significant differences in the VAF pre and post therapy (p=0.704) was observed. Notably, before starting BCL2i, the presence of non DNMT3A CHIP mutations associated with higher risk grade >3 neutropenia (p=0.04) during BCL2i. In addition, the relationship between CHIP and cardiovascular side effects was assessed in BTK inhibitors (BTKi) treated patients (N=73). CHIP+ SF3B1 mutated patients showed an increased risk of atrial fibrillation compared to SF3B1- patients (p<0.0001).

Conclusion
The analysis of the myeloid compartment in CLL patients suggests that CHIP may harbor potential clinical relevance in CLL and in Richter transformation. Single cell analyses are ongoing to deeply evaluate the interplay between CHIP and CLL.

P-11

New insights into the relationship between p53 and fak proteins in CLL

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Background
We previously found that the focal adhesion kinase (FAK) is present in the cleaved/phosphorylated and activated form in CLL patients, mainly IGHV unmutated (UM), through a mechanism involving other proteins that increases the aggressiveness of the disease. Activated FAK is present also in the cell nucleus where it might be directly involved in survival and proliferation. Studies in solid tumors highlighted how FAK could interact with p53. Since p53 can bind to FAK gene promoter and considering that TP53 is inactivated in about 15% of CLL patients accounted for unfavorable prognosis, the investigation on FAK-p53 interaction needs to be further explored.

Material and methods
CD19+/CD5+ cells from untreated CLL patients and normal B cells from age-matched healthy subjects were purified through density gradient centrifugation. Basal expression of p53, full-length (fl) FAK and cleaved (cl) FAK has been assessed by western blotting (WB) in 54 CLL patients and 10 controls. Nucleus-cytosol protein extraction has been performed in 7 CLL patients to analyze FAK and p53 subcellular distribution. p53 expression following FAK inhibition with 5 μM defactinib has also been assessed by western blotting at different time-points in 5 samples.

Results
WB analyses revealed a significant overexpression of p53 in patients versus controls ($p<0.0001$). Moreover, p53 showed to be higher expressed in UM-IGHV patients with respect to mutated ones ($p<0.05$). When we correlated p53 with activated cl-FAK, we found a significant positive correlation ($p<0.0001$, $r=0.57$), being p53 more expressed in patients with more active FAK, preferentially in UM ones. Accordingly, when we analyzed the mutual expression of FAK and p53 within the same CLL samples, we found that in the cohort of patients with p53high/fl-FAKlow there were mainly UM cases, differently from the cohort expressing p53high/fl-FAKhigh; in this latter group UM patients were less represented. In our case study we observed that p53 protein is mostly present in the nucleus of CLL cells (65% ± 0.05 of total protein) with also a fair amount of protein present in the cytosol (35% ± 0.05). Finally, we observed that defactinib is in some way able to modulate p53 expression in CLL; this latter aspect will require further investigation.

Conclusions
Our results provide a snapshot of the expression of p53 and FAK as well as their correlation in patients with CLL. Defactinib has previously been demonstrated to be more effective in unmutated patients and its action can modulate p53 expression, this indicating once again a possible interaction of FAK with p53 in CLL. The presence of both proteins in the nucleus of CLL cells is also interesting since FAK has been indicated as a scaffold protein for p53 itself.

P-12

Immunoglobulin light chain analysis refines the clinical impact of IGHV mutational status in chronic lymphocytic leukemia

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Background
The B-cell receptor (BCR) plays a pivotal role in chronic lymphocytic leukemia (CLL) pathogenesis. Between the two components of the BCR variable region, the Immunoglobulin heavy chain (IGHV) has been extensively studied and its mutational status not only serves as a robust prognostication tool but also represents a predictive biomarker for therapeutic choices. In contrast, the clinical relevance of the light chain gene recombination and mutational status in CLL is still largely unexplored. This study aimed to elucidate the prognostic role of light chain genes and their mutational status.

Material and methods
A real-life training-validation approach was used in this study. The light chain repertoire was analyzed by Sanger and/or by NGS sequencing methods.

Results
In the training cohort made up of 573 CLL patients (median follow-up 11.6 years), their characteristics were consistent with unselected CLL. A total of 530 productive rearrangements were identified, in which the most frequently rearranged kappa gene was IGKV4-1 in 84 patients, that comprised 20.5% of the total kappa rearrangements. Among lambda rearrangements, the most frequent one was IGLV3-21 (19%), out of which 59.4% harbored the R110 somatic point mutation. The IGLV3-21 and IGLV3-21R^{110} rearrangements associated with IGHV3-21 genes, borderline IGHV mutational status, subset#2, and SF3B1 mutations (all p<0.001). Patients with IGKV1-39 gene rearrangement associated with NOTCH1 mutations, trisomy 12, subset#1 and subset#8 (all p<0.001). By evaluating the risk of Richter transformation, the presence of IGKV1-39, IGKV6-21, IGLV1-36 and IGLV8-61 rearrangements (HR=4.89, 95% CI 1.26-18.94, p=0.02) was associated with a higher risk of Richter transformation, which was also confirmed in a multivariate analysis adjusted for NOTCH1 mutations and subset #8. A recursive partitioning approach identifies 99.0% of homology as the best cut-off that maximizes the log-rank statistics for TTFT in 414 Binet A CLL. Unmutated (UM) light chain patients (homology ≥99.0%) associated with shorter TTFT, with a 10-year probability of 32.4% compared to 73.2% for mutated (homology <99.0%) light chain patients (p<0.001). A multivariate analysis adjusted for the International Prognostic Score for Early-stage CLL (IPS-E) variables was performed and revealed that both UM light chains (HR=2.24, 95% CI 1.49-3.36, p<0.001) and UM-IGHV (HR=2.02, 95% CI 1.33-3.05, p<0.001) maintained an independent association with shorter TTFT. The prognostic role of light chain mutational status in terms of TTFT using the established homology cut-off of 99.0% was validated in an independent cohort that comprised 299 Rai 0 CLL patients. In addition, by combining the two cohorts (673 early stage CLL), UM light chains independently predicted shorter TTFT (HR=1.69, 95% CI 1.18-2.41, p=0.004) in a multivariate analysis when adjusted for the IPS-E variables.

Conclusion
In conclusion, this study represents the largest real-world cohort of unselected CLL analyzed for the immunoglobulin light chain gene repertoire. The mutational status of light chain genes refines the clinical impact of IGHV mutational status and independently predicts shorter TTFT in early stage CLL.

P-13

Dissecting the role of retinoic acid signaling in human chronic lymphocytic leukemia using novel 3D lymph node-like microenvironments

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Background
The stromal microenvironment of lymphoid tissue plays a central role in orchestrating adaptive immune responses. In lymphoid malignancies, such as chronic lymphocytic leukemia (CLL) and lymphomas (DLBCL, FL), stromal cells, also known as fibroblastic reticular cells (FRCs), are thought to nourish tumor cells by providing signals for survival, activation and proliferation. Using mouse models in conjunction with transcriptomics and in vitro stroma-leukemia cocultures, we have previously identified a retinoic acid (RA)-dependent stroma-leukemia crosstalk that promotes CLL progression. We also demonstrated that pharmacological inhibition of RA signalling alters the expression of genes associated with adhesion, tissue organization and chemokine secretion, and reduces infiltration of CLL cells into lymphoid tissues. Here, we further investigated the expression of RA-associated genes in human CLL lymph node biopsies and evaluated the role(s) of RA signalling in human stroma-leukemia crosstalk.

Materials and methods
For our studies we have employed conventional 2D cultures and 3D in vitro lymph node (LN)-like microenvironments using lymph node fibroblasts and primary human CLL cells, RNA-scope, flow cytometry and confocal microscopy.

Results
Using RA reporter mice, we observed that RA signalling increases during CLL progression. Furthermore, RNA-scope analysis revealed higher expression of genes involved in RA synthesis, degradation and signaling in human CLL lymph node biopsies compared to control tissues. We focused on RXRα, an RA nuclear receptor that we previously found to be overexpressed in human CLL cells, and tested its inhibition using conventional 2D cocultures and 3D models. Our results showed that inhibition of RXRα reduces the adhesion of CLL cells in 2D cultures and the aggregation of CLL cells to stromal cells in a humanized 3D spheroid model. To further validate these results under more physiological conditions, we developed a 3D LN microenvironment in a bioreactor system. We show that this model maintains the long-term viability of CLL cells and protects them from apoptosis. Using this system, we found that targeting RXRα interferes with interactions between stroma and leukemia and promotes the mobilization of leukemic cells from the 3D lymphoid microenvironment.

Conclusions
Our results show that the RA pathway is upregulated in human CLL biopsies and suggest that targeting RA nuclear receptors may represent a novel therapeutic strategy to interfere with stroma-leukemia interactions and promote mobilization of CLL cells from their protective immune niches.

P-14

Metabolic shift towards oxidative phosphorylation and glycolysis during Richter's syndrome transformation

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Background
Richter's syndrome (RS) represents the acute transformation of chronic lymphocytic leukemia (CLL) into an aggressive lymphoma, with a poor prognosis. Despite recent advancements in understanding the genetic and molecular mechanisms underlying the disease, the pathogenesis of RS remains poorly defined. This study aims to elucidate the metabolic landscape of RS cells and their substrate dependencies with the final goal of identifying potential weak features of these cells to be exploited in a translational perspective.

Material and methods
To address these aims, a cohort of primary CLL and RS samples, four patient-derived xenograft (PDX) models, and the RS cell line U-RT1, were exploited. Specifically, the expression and activity of key enzymes involved in glucose, glutamine, and fatty acid metabolisms were studied. Measurements of glucose and glutamine uptake were conducted using flow cytometry, oximetric, and luminometric assays, while untargeted metabolomic analysis was used to identify the main metabolites within RS cells. The metabolic dependencies of RS cells were assessed through in vitro treatments with selective metabolic inhibitors (BPTES, UK5099, and Etomoxir) and by evaluating oxidative phosphorylation and glycolysis in live cells (Agilent Seahorse XFe24 Analyzer), in the presence of selective inhibitors targeting PI3K and NF-kB.

Results
Transcriptomic data from primary CLL and RS samples and RS-PDX-derived cells shows a clear separation between the two disease phases and highlights cellular metabolism as the most significant differentially expressed term. Measurement of the activity of key enzymes confirmed the metabolic rewiring of RS cells, characterized by an elevated rate of Krebs cycle, oxidative phosphorylation, and glutamine metabolism. These pathways were sustained by increased glucose and glutamine uptake, two substrates these cells rely on. In line with these findings, a significant reduction in oxygen consumption rate and ATP production was observed when RS cells were treated with UK5099, a mitochondrial pyruvate carrier inhibitor, and BPTES, a glutaminase inhibitor, in turn resulting in substantial induction of apoptosis. Besides catabolic pathways, RS cells showed activation of anabolic processes that resulted in synthesizing building blocks necessary to sustain Finally, interference with PI3K and NF-kB, two key regulators of metabolic pathways, via selective inhibitors, markedly affected energy production through glycolysis and oxidative phosphorylation.

Conclusions
Overall, these data provide novel insights into the biology of the disease, describing the metabolic features of RS cells and their reliance on glucose and glutamine. These findings suggest that metabolic reprogramming occurs during the transition from CLL to RS and highlight metabolism as a potential therapeutic target for RS, either through direct and indirect inhibitors.

P-15

NOTCH1 orchestrates metabolic reprogramming to drive proliferation in a novel cellular model of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by the monoclonal expansion and accumulation of mature B cells in the peripheral blood and secondary lymphoid organs. Despite significant improvements in care with the introduction of targeted therapies, CLL remains incurable, and patients often relapse or become refractory to therapy. Signaling via surface immunoglobulins, as well as numerous genetic alterations, among which hyperactivating NOTCH1 mutations are the most frequent ones, play a crucial role in CLL pathogenesis and progression. The interplay between these signaling pathways and their effects on cellular pathobiology, however, remains incompletely understood. The objective of this study is to dissect the role of NOTCH1 in CLL and investigate whether its crosstalk with B cell receptor (BCR) activation bears functional consequences, with a specific focus on metabolic reprogramming. To do so, we developed a novel CLL cellular model by transducing a surface-IgM-negative subclone of the MEC-1 CLL-like cell line with a lentiviral vector expressing a patient-derived IGHV, thus exogenously reconstituting surface IgMs, in both a wild-type and a mutated NOTCH1 genetic background. We then performed bulk RNAseq on stimulated and unstimulated cells to unbiasedly investigate the transcriptional changes in our models. Subsequently, we characterized their metabolic profiles across genotypes, using various in vitro assays and isotope-tracing metabolomics. Lastly, we investigated whether metabolic reprogramming observed in our cellular models also occurred in primary CLL cells by performing Seahorse-based bioenergetics analyses. Bulk transcriptomics revealed that glycolysis, mitochondrial metabolism, and cell proliferation signatures are all upregulated in NOTCH1-mutated cells compared to wild-type, establishing NOTCH1 mutational status as the main driver of gene expression modulation. Using a variety of in vitro metabolic assays, we then confirmed the functional consequences of these transcriptional changes. NOTCH1-mutated cells exhibited higher glycolytic and oxidative metabolism, both basally and upon BCR stimulation, and proliferated at an increased rate in an in vitro competition assay. Isotopic tracer analysis of the metabolic fluxes identified an increased reliance on glutamine and more active anabolic pathways (i.e., pentose and amino acid biosynthesis) in NOTCH1-mutated cells. Lastly, we confirmed that primary CLL lymphocytes recapitulate the metabolic phenotypes observed in our cellular models. In conclusion, NOTCH1 hyperactivation, both in our cellular models and in patient-derived PBMCs, drives metabolic reprogramming and induces a shift towards glutamine-dependent pathways, supporting proliferation by strengthening the pentose shunt and amino acid biosynthesis. In the emerging framework of precision medicine, CLL patients could be stratified and assigned to therapeutic regimens exploiting the metabolic vulnerabilities associated with NOTCH1-mutated CLL.

P-16

Defining common genomic and transcriptomic profiles in Richter's syndrome paves the way for drug repurposing strategies

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Background
Richter's syndrome (RS) is a rare and aggressive lymphoma that occurs in patients with chronic lymphocytic leukemia (CLL). Despite advances in treatment, RS remains a challenging disease, with poor outcomes and limited therapeutic options. The purpose of this study is to analyze paired genomic and transcriptomic profiles of a cohort of RS patients.

The main goals are
i) expanding the understanding of RS genetic alterations that may contribute to disease transformation; ii) identifying the pathways that can be impacted/altered as consequence of genetic alterations; iii) defining common genomic and transcriptomic features of RS by leveraging different published patient cohorts; iv) suggesting potential druggable targets based on drug repurposing strategies.

Methods
DNA and RNA were extracted from a cohort of 20 formalin-fixed paraffin-embedded RS samples. DNA sequenced data were analyzed to identify disease-causing genetic variants, including single-nucleotide (SNV) and copy number variation (CNV), both in coding and non-coding regions. All identified variants were classified and annotated following the American College of Medical Genetics and Genomics criteria. ReMap, Jaspar and miRNASNP-v3 computational tools were used to assess the impact of non-coding variants on transcription factor binding sites (TFBSs), gene regulatory regions, including the 5'/3'-UTRs. Transcriptomic data were analyzed to highlight differentially expressed genes, comparing in-house generated data to publicly available ones, obtained both in CLL and RS cohorts. Gene ontology analysis was performed to define the biological processes of the differentially expressed genes. Moreover, drug repurposing strategies were proposed using pathway-based and target-based approaches, guided by genomic and transcriptomic analyses and the comparison with the publicly available RS cohorts.

Results
Genetic analysis confirmed the presence of recurrent mutations in RS patients, such as TP53 (45% of patients) and NOTCH1 (5% of patients). Moreover, novel lesions in genes - including LAMP1, PPM1D, and RECQL4 - implicated in the interferon-gamma response, IL-6/STAT3/JAK signaling, and p53 pathway were also identified. An analysis of non-coding variants showed two common intronic AKT2 variants that may disrupt TFBSs, as well as different 5'/3'-UTR variants impacting cell cycle and apoptosis. Several CNVs were outlined, affecting critical pathways such as G2/M checkpoint and the PI3K/AKT cascade. Ultimately, transcriptomic analysis revealed upregulation in genes related to DNA repair, cell cycle and cellular metabolism, and downregulation in ribosomal subunits and RNA processing.
Finally, the integration of genomic and transcriptomic data from both this study and recently published cohorts allowed for the identification of pathways that can be targeted with FDA-approved or phase III/IV drugs. The most promising pathways included apoptosis, cell proliferation, metabolism, DNA repair, and proinflammatory signalling.

Conclusion
Overall, these results lead to the identification of novel genetic lesions, including non-coding variants potentially contributing to RS pathogenesis. Novel mechanisms underlying the disease, such as the downregulation of ribosomal subunits or potential drug resistance, were highlighted. Comparative analyses across RS cohorts revealed common genomic and transcriptomic signatures and allowed us to speculate on potential drug repurposing strategies.

P-17

Elevated 5-hydroxymethyluracil in CLL patients: implications for protein expression

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Background
Modified DNA bases, such as product of methylation/demethylation/deamination processes, play a crucial role in regulating gene expression by altering the accessibility of DNA to transcriptional machinery. These modifications can influence protein expression patterns and are key in processes such as development, cellular differentiation, and disease.

Materials and methods
The study recruited patients diagnosed with CLL with stable disease who did not require treatment, along with the control group. We determined the levels of several DNA modifications involved in the epigenetic regulation and products of its repair in urine using 2D-UPLC-MS/MS (Acquity UPLC and TQ-XS or Xevo TQ-S, Waters). Based on the results obtained, patients were stratified into 3 groups: low, medium, and high content of 5-hydroxymethyluracil (5hmU) in DNA, with 25 people in each group. Then, a proteomic screening study of blood plasma was performed using LC-MS/MS (Dionex Ultimate3000-Exploris 480, Thermo Fisher Scientific).

Results
Among the determined modifications, (5hmU) had several times higher concentrations in CLL than in healthy people. Similarly, CLL patients had higher levels of urinary 5hmU excretion than the control group. Moreover, a high level of this modification correlated with a shortened time-to-treatment in CLL patients in the Kaplan-Meier analysis. To assess how these differences could translate into the disease phenotype, we decided to investigate whether there are differences in protein expression in patients with different levels of 5hmU. As a result of the analysis, 76 differentially expressed proteins were identified, 24 of which displayed significantly different protein abundance in at least 2 groups of 5hmU. The largest differences were observed in the case of 8 proteins that showed a positive (SERPINA6, SELL, LRG1, B2M, C7) or negative (CLIC1, BTD, DEFA1) correlation with the amount of 5hmU. Additionally, 26 immunoglobulins were identified, the majority of which were inversely proportional to the level of 5hmU.

Conclusion
Our results revealed a higher content of 5hmU in the DNA of CLL patients. Moreover, we have shown that this level varies significantly. It is possible, that a high amount of 5hmU in DNA affects gene expression and translates into the concentrations of some proteins circulating in the blood plasma of CLL patients. These observations raise further questions about the impact of 5hmU on the mechanisms regulating gene expression and their consequences in the development of CLL.

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P-18

Influence of gut microbiota profile on the response to MRD-driven venetoclax and rituximab therapy in chronic lymphocytic leukemia - a pilot study

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Background
Minimal residual disease (MRD) status after venetoclax treatment in chronic lymphocytic leukemia (CLL) is an important predictive factor of progression-free survival (PFS). Nevertheless, biological markers of achieving MRD negativity are limited. Gut microbiota (GMB) has a well-studied influence on the disease progression and outcomes of treatment in lymphoid malignancies. Dysbiosis was a prognostic factor of decreased PFS in CLL after chemoimmunotherapy. In multiple myeloma, Eubacterium hallii correlated with MRD(-). However, the role of GMB in the context of response to targeted therapy in CLL remains unclear. The study aimed to correlate the pretreatment GMB composition with the status of MRD in CLL patients treated with first line MRD-driven venetoclax and rituximab (VR) combination given within ongoing VERITA PALG-CLL5 trial.

Material and methods
As per VERITA PALG-CLL5 study protocol fecal samples were collected prior to the start of VR therapy from all 103 patients included to the study. For this pilot study, only patients who had completed 12 cycles of VR therapy and had assessed MRD status were selected. MRD was evaluated in peripheral blood and bone marrow aspirate via flow cytometry. Bacterial DNA sequencing was performed with Illumina NovaSeq 6000 technology. Taxonomical profiling was done using the QIIME, PATRIC, and other tools. Alpha diversity analysis and Shannon diversity and richness index were used to compare patients with MRD(+) vs MRD(-). Principle component analysis (PCA) was used to describe the gut microbial profile, with taxa having the main impact on the sample clustering.

Results
A total of 17 patients with known MRD status were enrolled. The median age was 59 (range, 42-77), and 10 (59%) patients were male. Del(17p) and/or TP53 mutations were present in 3 patients, while 9 patients had unmutated IGHV. Regarding treatment outcomes at 12 months, ten patients (59%) achieved complete response with MRD(-). Forty-nine species were identified in the cohort's fecal samples. Shannon alpha diversity was numerically higher in MRD+ (median 5.12, IQR 4.59-5.56) compared to MRD- (median 4.86, IQR 4.68-5.34), however, no statistical significance was revealed (p=0.758, Mann-Whitney test). MRD(+) subgroup was substantially characterized by higher GMB richness (p=0.023, Mann-Whitney test) compared to MRD(-) patients. Analysis of bacterial species abundances in microbiomes of MRD(-) and MRD(+) patients demonstrated a visible separation in the first and second principal components. Twelve species were mainly responsible for this effect. Prevotella copri and Bifidobacterium adolescentis were the most different taxa among the subgroups and were observed more abundantly in the MRD(+) subgroup.

Conclusions
Our preliminary results imply that GMB may have some influence on the probability of achieving an MRD(-) response in CLL with venetoclax-based treatment. Some species, like Prevotella copri and Bifidobacterium adolescentis, were more abundant in MRD(+) patients, suggesting their possible involvement in MRD status clearance. Further studies on larger cohorts are essential to confirm these early observations.

P-19

Efficacy and toxicity of venetoclax and obinutuzumab in the first-line CLL patients with comorbidities: analysis of real-world data from the Polish Adult Leukemia Group (PALG) centers

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Background
Venetoclax combined with obinutuzumab (VG) is considered the standard of care for patients with untreated chronic lymphocytic leukemia (CLL) and comorbid conditions based on the results of CLL14 phase 3 clinical trial. The present study aimed to analyze the efficacy and toxicity of VG in a real-world population of comorbid patients treated in the centers of the Polish Adult Leukemia Group (PALG) outside clinical trials.

Material and methods
This was a retrospective analysis of adult patients with untreated CLL and comorbid conditions defined as a Cumulative Illness Rating Scale (CIRS) total score of >6 or a creatinine clearance (CrCl) < 70mL/minute treated within CLL Therapeutic Programme of the Polish National Health Fund. A log-rank test was used for group comparison.

Results
A total of 220 CLL patients treated with first-line VG were included. The median age at VG commencement was 70 years (range: 45-86), and 134/220 (60.9%) were male. The median time between diagnosis and treatment was 1.6 year (range: 0-19). The median ECOG was 1 (range: 0-3). The median CIRS score was 8 (range: 0-30), and 101 (45.9%) patients had CrCL<70 ml/min. TP53 aberration (del17p and/or TP53 mutation) was detected in 19 (9.9%) patients out of 191 tested individuals. CLL stage, according to Rai, was ≥3 in 111/220 (50.4%) patients. Tumor lysis syndrome (TLS) risk was intermediate in 102/203 (50.2%) and high in 69/203 (34%). At the time of analysis, 86/220 (39%) patients were continuing treatment, and 134/220 (61%) completed the therapy. In that group, 98/134 (73.9%) patients completed treatment as per protocol. The main reasons for treatment discontinuation were adverse events (AEs), most commonly infections, liver toxicity, and second primary malignancies (SPM). Clinical TLS was observed in 11/203 (5.4%) patients, while biochemical TLS was seen in 41/203 (20.2%) patients. The overall response rate for evaluable patients was 92%. After a 12-month median follow-up (95% CI: 11.8-13.8), progression-free survival (PFS) was 92.7% (95% CI: 89-97), while overall survival (OS) was 93.2% (95% CI: 89.5%-97.0%). Harboring del17p/TP53 impacted neither PFS (p=0.96) nor OS (p=0.94). Some differences were observed when comparing patients with comorbidities (CIRS>6 vs. CIRS<6); however, OS (p=0.09) and PFS (p=0.08) did not significantly differ. Hematological toxicity of any grade was the most predominant, with 79.5% of patients developing neutropenia, 55.9% thrombocytopenia, and 52.3% anemia. The other most frequent AEs were COVID19 (27/201, 13.4%), infusion-related reactions (10.5%), elevated liver enzyme activity (8.2%), pneumonia

(8.2%), neutropenic fever (8.2%) and upper respiratory tract infection (7.3%). Autoimmune phenomena occurred in 8 (3.6%) patients, comprising 7 autoimmune hemolytic anemias and 5 immune thrombocytopenia. There were 8 SPM in the analyzed cohort. Thirteen (5.9%) patients died. The leading causes of death were infections (4 patients), CLL progression (3 patients) and SPM (2 patients).

Conclusions

VG combination is effective and well-tolerated in patients with CLL and comorbidities in real-world conditions. In our relatively short observation TP53 aberrations do not seem to impact significantly the early treatment outcomes, though extended follow-up is mandatory.

P-20

Health-related quality of life in patients with relapsed / refractory chronic lymphocytic leukemia improves after treatment with venetoclax plus ibrutinib

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Background
Maintaining and improving health-related quality of life (HRQoL) is a crucial treatment objective for patients with chronic lymphocytic leukemia (CLL) due to the incurable nature of the disease. The HOVON 141/Vision trial has demonstrated a favorable benefit-risk profile for MRD-guided, time-limited treatment with ibrutinib plus venetoclax in patients with relapsed or refractory (RR) CLL. However, data on the effect of ibrutinib plus venetoclax on HRQoL are scarce.

Materials and Methods
Assessment of HRQoL in patients with RR CLL was conducted within the framework of the HOVON 141/Vision study as a pre-planned, post-hoc analysis. Specifically, the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire CLL-specific 16 (EORTC QLQ-CLL16) were used. Patients completed these questionnaires at baseline and after 15 cycles of ibrutinib plus venetoclax. Minimal important differences were used to determine whether changes in scores represent clinically meaningful improvements or deteriorations.

Results
A total of 224 patients who completed at least one questionnaire were included in the analysis. The majority of patients were male, with a median age of 68 years (IQR 61-73). At baseline, the median global health status was 66, measured on a scale from 0 to 100, with 100 representing a perfect health status. Clinically relevant improvement of 9,6 points was seen in the global health status, with a median global health status after 15 cycles of ibrutinib plus venetoclax of 75. Similarly, a clinically relevant decrease of in disease-related symptoms (-10) and future health concerns (-12) was observed based on the EORTC QLQ-CLL16 assessment. There was an increase in treatment-related symptoms (+8), such as diarrhea (+14). When comparing baseline scores to scores after 15 cycles of ibrutinib plus venetoclax on a patient level, 44% of patients showed a clinically relevant improvement in global health status, while 41% had a stable score for global health status. Only 15% showed a clinically relevant decrease in global health status. The majority of patients (79%) experienced one or more grade 3-5 adverse event during induction therapy. However, there was no correlation between grade 3-5 adverse events during induction therapy and the global health status.

Conclusion
Treatment with ibrutinib plus venetoclax significantly improves HRQoL in RR CLL patients by reducing disease-related symptoms and future health concerns. Treatment with ibrutinib plus venetoclax is associated with an increase in treatment-related symptoms, such as diarrhea. However, the overall effect of the treatment is positive, as evidenced by a clinically relevant increase in global health status for the majority of patients.

P-21

TP53-related clonal competition and disrupted molecular processes in CLL

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Background

The presence of either TP53 gene loss or mutation defined high-risk CLL especially in the era of chemoimmunotherapy. Its prognostic and predictive impact remains a matter of debate in the setting of BTKi- and BCL2i-based treatment, which does not act directly via the TP53 pathway, but yet seems to associate with worse clinical outcomes. The adverse effect on overall survival, at least upon chemoimmunotherapy, is notable not only in patients with expanded but also in low-burden TP53 mutations, which were identified in 5–35% of CLL patients. In this study, we aimed to scrutinize the principles of clonal competition in CLL and identify factors influencing the development of TP53-mutated subclones in various clinical contexts.

Material and methods

We investigated a cohort of 62 CLL patients with known patterns of TP53 mutation evolution. The patients were treated and monitored at the University Hospital Brno, with a median interval between the first and last sample of 71 months (range 21–183). Whole-exome sequencing was employed to identify somatic variants at two to six different timepoints during the disease course of each patient. We mapped somatic variants to the respective molecular pathways and studied their disruption under different TP53 mutation behavior over time. Additionally, we analyzed the mutational signatures suggestive of different mutational processes. In a subset of cases, RNA sequencing was performed. The abnormalities observed on the DNA level were further explored in the expression data.

Results

The expansion of TP53 defects was observed upon different therapy regimens and different number of therapy lines. The most prevalent scenario was acquiring or further expanding the TP53 defect upon FCR in the first line in agreement with our published findings. However, many cases did not undergo TP53 defects' expansion even when they received therapy with strong selection pressure; they had shorter progression-free survival than patients with the defects expanding upon the same therapy (7 months less on average). In those patients without TP53 defects' selection, we found subclones with disrupted proteolysis or apoptosis pathways, often present already in treatment-naïve samples. This suggests a potential high-risk group, as patients with impaired apoptosis may not benefit from treatment with BCR signaling inhibitors in the first line either. On top of that, we performed a detailed analysis of cases harboring multiple concomitant TP53 defects (≥10 mutations) and suggested potential causative activity of activation-induced cytidine deaminase (AID).

Conclusions

Focusing on alterations in whole gene sets (i.e., molecular pathways) or mutational signatures rather than single gene aberrations can be very efficient, especially in diseases with large genomic heterogeneity, such as CLL, where gene-based analysis alone would show no differences. Our study revealed impaired pathways and patterns of mutational mechanisms associated with different disease stages, treatment outcomes, and TP53 status. We observed that the selection of TP53-mutated subclones is often compensated by subclones harboring other defects with similar functional impact.

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